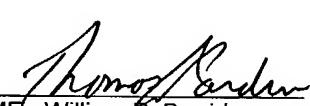


(1390 REV. 5-93) US DEPT. OF COMMERCE PATENT & TRADEMARK OFFICE  <b>TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371</b>		ATTORNEY'S DOCKET NUMBER 111968
		U.S. APPLICATION NO (if known, sec 37 C.F.R.1.5)
		<b>10/049552</b>
INTERNATIONAL APPLICATION NO. PCT/FR99/02043	INTERNATIONAL FILING DATE August 26, 1999	PRIORITY DATE CLAIMED
TITLE OF INVENTION USE OF BACTERIAL EXTRACTS FROM THE FAMILY PSEUDOMONADACEAE AS COSMETIC AGENTS		
APPLICANTS FOR DO/EO/US Martin RICHARD, Pascal HILAIRE, Nathalie PINEAU, Lionel BRETON		
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:		
<ol style="list-style-type: none"> <li>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li>3. <input checked="" type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).</li> <li>4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.</li> <li>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2))             <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).</li> <li>b. <input checked="" type="checkbox"/> has been transmitted by the International Bureau.</li> <li>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US)</li> </ol> </li> <li>6. <input checked="" type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).</li> <li>7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))             <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).</li> <li>b. <input type="checkbox"/> have been transmitted by the International Bureau.</li> <li>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</li> <li>d. <input type="checkbox"/> have not been made and will not be made.</li> </ol> </li> <li>8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</li> <li>9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).</li> <li>10. <input checked="" type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).</li> </ol>		
<b>Items 11. to 16. below concern other document(s) or information included:</b>		
<ol style="list-style-type: none"> <li>11. <input checked="" type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</li> <li>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</li> <li>13. <input checked="" type="checkbox"/> A FIRST preliminary amendment.               <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.</li> <li>14. <input type="checkbox"/> A substitute specification.</li> <li>15. <input type="checkbox"/> Entitlement to small entity status is hereby asserted.</li> <li>16. <input type="checkbox"/> Other items or information:</li> </ol>		

U.S. APPLICATION NO. (if known, see 37 C.F.R. 1.5)	10/049552	INTERNATIONAL APPLICATION NO.	PCT/FR99/02043	ATTORNEY'S DOCKET NUMBER	111968
17. <input checked="" type="checkbox"/> The following fees are submitted:			CALCULATIONS	PTO USE ONLY	
<b>Basic National fee (37 CFR 1.492(a)(1)-(5)):</b>					
Search Report has been prepared by the EPO or JPO ....\$890.00					
International preliminary examination fee paid to USPTO (37 CFR 1.482) .....\$710.00					
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) .....\$740.00					
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO .....\$1,040.00					
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) .....\$ 100.00					
<b>ENTER APPROPRIATE BASIC FEE AMOUNT = \$890.00</b>					
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).					
Claims	Number Filed	Number Extra	Rate		
Total Claims	20- 20 =	0	X \$ 18.00	\$	
Independent Claims	2- 3 =	0	X \$ 84.00	\$	
Multiple dependent claim(s)(if applicable)			+ \$280.00	\$	
<b>TOTAL OF ABOVE CALCULATIONS = \$890.00</b>					
Reduction by 1/2 for filing by small entity, if applicable.				-	\$
				<b>SUBTOTAL = \$890.00</b>	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 month from the earliest claimed priority date (37 CFR 1.492(f)).				\$	
				+ \$	
<b>TOTAL NATIONAL FEE = \$890.00</b>					
				Amount to be refunded	\$
				Charged	\$
a. <input checked="" type="checkbox"/> Check No. <u>127814</u> in the amount of <u>\$890.00</u> to cover the above fees is enclosed.					
b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.					
c. <input checked="" type="checkbox"/> The Director is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Deposit Account No. <u>15-0461</u> . A duplicate copy of this sheet is enclosed.					
<b>NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.</b>					
SEND ALL CORRESPONDENCE TO: OLIFF & BERRIDGE, PLC P.O. Box 19928 Alexandria, Virginia 22320					
 NAME: William P. Berridge REGISTRATION NUMBER: 30,024					
Date: <u>February 14, 2002</u> NAME: Thomas J. Pardini REGISTRATION NUMBER: 30,411					

0/049552  
JC13 Rec'd PCT/PTO 14 FEB 2002

**PATENT APPLICATION**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Martin RICHARD, Pascal HILAIRE, Attn: PCT Branch  
Nathalie PINEAU, Lionel BRETON

Application No. U.S. National Stage of PCT/FR99/02043

Filed: February 14, 2002 Docket No.: 111968

For: USE OF BACTERIAL EXTRACTS FROM THE FAMILY  
PSEUDOMONADACEAE AS COSMETIC AGENTS

**TRANSLATION OF THE ANNEXES TO THE  
INTERNATIONAL PRELIMINARY EXAMINATION REPORT**

Director of the U.S. Patent and Trademark Office  
Washington, D.C. 20231

Sir:

Attached hereto is a translation of the annexes to the International Preliminary Examination Report (Form PCT/IPEA/409). The attached translated material replaces the material in the claims.

Respectfully submitted,



William P. Berridge  
Registration No. 30,024

Thomas J. Pardini  
Registration No. 30,411

WPB:TJP/cmm

Date: February 14, 2002

OLIFF & BERRIDGE, PLC  
P.O. Box 19928  
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Telephone: (703) 836-6400

DEPOSIT ACCOUNT USE AUTHORIZATION Please grant any extension necessary for entry; Charge any fee due to our Deposit Account No. 15-0461
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CLAIMS

1. Use of an extract of at least one bacterium from the family Pseudomonadaceae, as a cosmetic agent intended to combat ageing of the skin, including photoageing, to improve the appearance and tonicity of dry skin, to preserve or improve skin elasticity, and/or to improve the appearance of skin which exhibits a local inflammatory reaction, said extract consisting either of the biomass obtained after culturing said bacterium, optionally followed by purification, grinding, at least partial dehydration and/or sterilization, or of a fraction or a derivative, obtained by chemical modification of certain functional groups, of said biomass, said fraction or said derivative having elastase activity inhibition and/or hyaluronidase activity inhibition properties.
2. Use according to Claim 1, in which said bacterium belongs to the genus *Pseudomonas*.
3. Use according to Claim 2, in which said bacterium is chosen from *Pseudomonas vesicularis* and *Pseudomonas maltophilia*.
4. Use according to any one of the preceding claims, in which said extract consists of the bacterial biomass obtained after culturing the bacterium, said biomass optionally being ground and/or at least partially dehydrated.
5. Use according to any one of the preceding claims, in which said extract is applied in the form of a composition containing a proportion of 0.0005% to

- 5% by weight of bacterial solids relative to the total weight of the composition.
6. Use according to the preceding claim, in which said proportion is within the range of 0.001% to 2% by weight.
  7. Cosmetic treatment method intended to combat ageing of the skin, including photoageing, to improve the appearance and tonicity of dry skin, to preserve or improve skin elasticity, and/or to improve the appearance of skin which exhibits a local inflammatory reaction, said method comprising the step consisting in applying an extract of at least one bacterium from the family Pseudomonadaceae to the skin or to the scalp, said extract either consisting of the biomass obtained after culturing said bacterium, optionally followed by purification, grinding, at least partial dehydration and/or sterilization, or of a fraction or a derivative, obtained by chemical modification of certain functional groups, of said biomass, said fraction or derivative having elastase activity inhibition and/or hyaluronidase activity inhibition properties.
  8. Method according to the preceding claim, having at least one of the following characteristics:
    - the bacterium belongs to the genus *Pseudomonas*;
    - the bacterium belongs to the species *Pseudomonas vesicularis* or *Pseudomonas maltophilia*;
    - said extract consists of the bacterial biomass obtained after culturing the bacterium, said biomass optionally being ground and/or at least partially dehydrated;

- said extract is applied in the form of a composition containing a proportion of 0.0005% to 5%, and in particular of 0.001 to 2%, by weight of bacterial solids relative to the total weight of the composition.
- 9. Method according to either of Claims 7 and 8, intended to combat photoageing of the skin.
- 10. Method according to either of Claims 7 and 8, intended to improve the appearance of dry skin.
- 11. Method according to either of Claims 7 and 8, intended to improve the tonicity of dry skin.
- 12. Method according to either of Claims 7 and 8, intended to preserve or improve skin elasticity.
- 13. Method according to either of Claims 7 and 8, intended to improve the appearance of skin which exhibits a local inflammatory reaction.
- 14. Cosmetic composition comprising, as an active ingredient, an extract of at least one bacterium from the family *Pseudomonadaceae*, in combination with an excipient which is acceptable in cosmetology, said extract consisting either of the biomass obtained after culturing said bacterium, optionally followed by purification, grinding, at least partial dehydration and/or sterilization, or of a fraction or a derivative, obtained by chemical modification of certain functional groups, of said biomass, said fraction or said derivative having elastase activity inhibition and/or hyaluronidase activity inhibition properties.

15. Composition according to Claim 14, having at least one of the following characteristics:
  - the bacterium belongs to the genus *Pseudomonas*;
  - the bacterium belongs to the species *Pseudomonas vesicularis* or *Pseudomonas maltophilia*;
  - said extract consists of the bacterial biomass obtained after culturing the bacterium, said biomass being at least partially dehydrated;
  - said composition contains a proportion of 0.0005% to 5%, and in particular of 0.001% to 2%, by weight of bacterial solids relative to the total weight of the composition.

10/049552  
PATENT APPLICATION  
JC13 Rec'd PCT/PTO 14 FEB 2002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Martin RICHARD, Pascal HILAIRE, Nathalie PINEAU,  
Lionel BRETON

Application No.: U.S. National Stage  
of PCT/FR99/02043

Filed: February 14, 2002

Docket No.: 111968

For: USE OF BACTERIAL EXTRACTS FROM THE FAMILY PSEUDOMONADACEAE  
AS COSMETIC AGENTS

PRELIMINARY AMENDMENT

Director of the U.S. Patent and Trademark Office  
Washington, D. C. 20231

Sir:

Prior to initial examination, but after entry of the annexes to the IPER, please amend  
the above-identified application as follows:

IN THE TITLE:

Please replace the title as follows:

USE OF BACTERIAL EXTRACTS FROM THE FAMILY PSEUDOMONADACEAE AS  
COSMETIC AGENTS

IN THE CLAIMS:

Please cancel claims 1-15 without prejudice to or disclaimer of the subject matter  
contained therein.

Please add new claims 16-35 as follow:

--16. A cosmetic treatment method applied to combat ageing of skin, including  
photoageing, to improve appearance and tonicity of dry skin, to preserve or improve skin  
elasticity, and/or to improve appearance of skin which exhibits a local inflammatory reaction,  
said method comprising a step of applying an extract of at least one bacterium from the

family Pseudomonadaceae to the skin or to the scalp, said extract either comprising a biomass obtained after culturing said bacterium, optionally followed by one or more members selected from the group consisting of purification, grinding, partial or complete dehydration and sterilization, or a fraction or a derivative, obtained by chemical modification of certain functional groups, of said biomass, said fraction or derivative having at least one of elastase activity inhibition and hyaluronidase activity inhibition properties.--

--17. Method according to claim 16, having at least one of the following features:

the bacterium belongs to the genus *Pseudomonas*;

the bacterium belongs to the species *Pseudomonas vesicularis* or *Pseudomonas maltophilia*;

said extract is comprised of a bacterial biomass obtained after culturing the bacterium, said biomass optionally being ground and/or partially or completely dehydrated;

said extract is applied in the form of a composition containing a proportion of 0.0005% to 5% by weight of bacterial solids relative to the total weight of the composition.--

--18. Method according to claim 16, in which said bacterium belongs to the genus *Pseudomonas*.--

--19. Method according to claim 18, in which said bacterium is at least one member selected from the group consisting of *Pseudomonas vesicularis* and *Pseudomonas maltophilia*.--

--20. Method according to claim 16, in which said extract is comprised of a bacterial biomass obtained after culturing the bacterium, said biomass optionally being ground and/or partially or completely dehydrated.--

--21. Method according to claim 16, in which said extract is applied in the form of a composition containing a proportion of 0.0005% to 5% by weight of bacterial solids relative to the total weight of the composition.--

--22. Method according to claim 21, in which said proportion is within the range of 0.001% to 2% by weight.--

--23. Method according to claim 16, applied to combat photoageing of the skin.--

--24. Method according to claim 17, applied to combat photoageing of the skin.--

--25. Method according to claim 16, applied to improve the appearance of dry skin.--

--26. Method according to claim 17, applied to improve the appearance of dry skin.--

--27. Method according to claim 16, applied to improve the tonicity of dry skin.--

--28. Method according to claim 17, applied to improve the tonicity of dry skin.--

--29. Method according to claim 16, applied to preserve or improve skin elasticity.--

--30. Method according to claim 17, applied to preserve or improve skin elasticity.--

--31. Method according to claim 16, applied to improve the appearance of skin which exhibits a local inflammatory reaction.--

--32. Method according to claim 17, applied to improve the appearance of skin which exhibits a local inflammatory reaction.--

--33. A cosmetic composition comprising, as an active ingredient, an extract of at least one bacterium from the family Pseudomonadaceae, in combination with an excipient which is acceptable in cosmetology, said extract comprising either a biomass obtained after culturing said bacterium, optionally followed by one or more members selected from the group consisting of purification, grinding, partial or complete dehydration and sterilization, or a fraction or a derivative, obtained by chemical modification of certain functional groups, of said biomass, said fraction or said derivative having at least one of elastase activity inhibition and hyaluronidase activity inhibition properties.--

--34. Composition according to claim 33, having at least one of the following features:

- the bacterium belongs to the genus *Pseudomonas*;
- the bacterium belongs to the species *Pseudomonas vesicularis* or *Pseudomonas maltophilia*;
- said extract is comprised of a bacterial biomass obtained after culturing the bacterium, said biomass being at least partially dehydrated;
- said composition contains a proportion of 0.0005% to 5% by weight of bacterial solids relative to the total weight of the composition.--

--35. Composition according to claim 34, wherein said proportion is within the range of 0.001% to 2% by weight.--

REMARKS

Following entry of the Annexes to the International Preliminary Examination Report (IPER) and the above amendments, claims 16-35 are pending. The above amendments are not presented for patentability, but rather to place the claims in a more appropriate form for U.S. examination, without narrowing of the claims. Prompt and favorable action on the merits of claims 16-35 is respectfully requested.

The attached Appendix includes a marked-up copy of the title.

Respectfully submitted,



William P. Berridge  
Registration No. 30,024

Thomas J. Pardini  
Registration No. 30,411

WPB:TJP/cmm

Attachment: Appendix

Date: February 14, 2002

**OLIFF & BERRIDGE, PLC**  
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**Telephone: (703) 836-6400**

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10/049552

Docket No. 111968

JC13 Rec'd PCT/PTO 14 FEB 2002  
U.S. National Stage of PCT/FR99/02043

## APPENDIX

Changes to Title:

The following is a marked-up version of the amended title:

USE OF BACTERIAL EXTRACTS FROM THE FAMILY PSUEDOMONADACEAE AS  
COSMETIC AGENTS OF THE PSEUDOMONADACEAE FAMILY AS COSMETIC  
AGENTS

P.C.T. PATENT APPLICATION  
Filed on: 26 AUGUST 1999  
Under the No. PCT/FR99/02043

IN ACCORDANCE WITH THE FILING

Use of bacterial extracts from the family  
Pseudomonadaceae as cosmetic agents

The invention relates to the use of extracts of  
5 bacteria from the family Pseudomonadaceae, as cosmetic  
agents for in particular combating ageing of the skin  
in humans.

It is known that ageing of the skin manifests itself,  
10 firstly, through a decrease in the number and a  
fragmentation of the elastic fibres of the dermis.  
Elastin becomes more sensitive to lysis by elastase and  
the deterioration of the elastin leads to  
disorganization of the elastic fibres. These phenomena  
15 result in a loss of the elasticity of the skin and in  
the formation of wrinkles.

Another manifestation of ageing of the skin is dryness  
of the skin, which becomes rough, with a loss of  
20 flexibility of the epidermis and a tendency towards  
desquamation. In the connective tissue of the skin of  
young individuals, the high content of hyaluronates,  
which are highly hydrophilic, promotes moisturization  
of the dermis, which is an essential element of skin  
25 tonicity. During ageing, the hyaluronate content, and  
therefore the water content, of the dermis greatly  
decreases, with the unfortunate consequences that the  
skin is flaccid and there is less diffusion of dermal  
water to the epidermis, which dries out. In addition,  
30 as a consequence of the decrease in the water content  
of the dermis, the circulation of metabolites, of ions  
and of oxygen is in particular impeded and, therefore,  
the metabolism of the dermal and epidermal cells slows  
down. The decrease in the hyaluronate content is linked  
35 to the activity of an enzyme, hyaluronidase, which  
cleaves the glycosidic bonds of hyaluronates. For this

N ACCORDANCE WITH THE FILING

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reason, this enzyme plays a very important role in ageing of the skin.

In addition, the drying out of the epidermis decreases  
5 the gaseous exchanges with the ambient atmosphere at  
the surface of the skin. This phenomenon of gaseous  
exchange, called cutaneous respiration, decreases with  
age.

10 Moreover, it is known that exposure to sunlight may cause an inflammatory reaction in skin tissue and that, after repeated and prolonged exposure to sunlight, in particular to UVA radiation, the skin eventually becomes dried out, excessively wrinkled and lacking in 15 flexibility: this premature ageing of the skin is called "photoageing".

It is therefore desirable to find new means in particular for protecting the skin against accelerated or premature ageing, and for more effectively protecting the skin against damage caused by exposure to sunlight, including against photoageing of the skin.

It has now been discovered that extracts of bacteria from the family Pseudomonadaceae, and in particular bacteria of the genus *Pseudomonas*, when applied to the skin, are in particular capable of improving moisturization of the skin and protecting the skin against certain harmful consequences of inflammatory reactions subsequent to exposure to ultraviolet radiation. More generally, they are capable of decreasing and/or delaying ageing of the skin, including photoageing of the skin.

35 These bacterial extracts have in particular the  
property of inhibiting lesions of the connective tissue  
of the skin subsequent in particular to UV exposure.  
These bacterial extracts in fact have the property of

IN ACCORDANCE WITH THE FILING

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- inhibiting elastase release in areas of inflammation, as shown in the experimental section hereinafter, and they also have the property of inhibiting elastase activity. More generally, these bacterial extracts, 5 when applied to the skin, have anti-inflammatory and soothing properties, and improve the appearance of skin which exhibits a local inflammation or micro-inflammations, including after exposure to sunlight.
- 10 These bacterial extracts also have an inhibitory effect on hyaluronidase activity. Thus, they make it possible to prevent or treat dryness of the skin, including after exposure to sunlight and in cases of natural or premature ageing of the skin, and also in cases of 15 photoageing. In addition, they improve skin tonicity by promoting moisturization of the dermis.

A subject of the invention is therefore the use of an extract of at least one bacterium from the family 20 Pseudomonadaceae, as a cosmetic agent for combating natural or premature ageing of the skin, including photoageing, for improving the appearance and tonicity of dry skin, for preserving or improving skin elasticity, and/or for improving the appearance of skin 25 which exhibits an inflammatory reaction, including after exposure to sunlight. In the present application, the expression "combating" ageing of the skin means preventing or delaying, or even treating, ageing of the skin.

30

Among the bacteria which may be used according to the invention, mention may be made in particular of:

- *Pseudomonas vesicularis*, one of the types of which 35 is the strain deposited at the ATCC under the No. 11426.

IN ACCORDANCE WITH THE FILING

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- *Pseudomonas maltophilia*, one of the types of which is the strain deposited at the ATCC under the No. 13637.

5 *Pseudomonas maltophilia* is also called *Stenotrophomonas maltophilia*.

In the present application, the expression "extracts of bacteria" or "bacterial extracts" denotes both the biomasses obtained after culturing the bacteria and the products obtained from these biomasses, in particular after purification and/or sterilization and/or fractionation. For example, the biomasses may optionally be at least partially dehydrated and/or ground. They may be sterilized, for example by heating. Of course, the invention extends to the use of extracts comprising any fraction of the biomass which has the same anti-ageing of the skin properties as the whole biomass, and in particular fractions which inhibit elastase secretion in areas of inflammation and/or 15 which inhibit hyaluronidase activity. In the present application, the notion of extracts also encompasses derivatives obtained by chemical modification of 20 certain functional groups (amines for example).

25 The method for preparing a bacterial extract used according to the invention comprises the steps consisting in culturing, *in vitro*, the bacteria according to known methods and then in collecting the biomass obtained.

30 Bacteria of the family Pseudomonadaceae are strictly aerobic Gram-negative bacteria. They grow on ordinary nutrient media, for example at temperatures of the order of 25 to 30°C.

35 To separate and isolate the biomass, various known methods, such as filtration or centrifugation, may be

- 5 -

used. It is also possible to dry the biomass and concentrate it by dehydration, in particular by heating under reduced pressure (for example at a temperature of the order of approximately 80 to 120°C) or by 5 lyophilization.

The bacterial extracts may be used in the form of derivatives, for example of at least partially acylated derivatives. The acylation is carried out using a 10 carboxylic acid anhydride or with a corresponding acid chloride. Use may be made, for example, of acetic anhydride or acetyl chloride. The acylation reaction is carried out such that at least some of the primary and 15 secondary amine groups present in the bacterial biomass are acylated. The proportions of acylating agents and the acylation reaction conditions are easily determined by assaying, according to conventional methods, the primary and secondary amine groups before and after the acylation reaction.

20

The extracts of *Pseudomonadaceae*, or derivatives thereof, are introduced, as active ingredients, into compositions intended to be applied to the skin and/or to the scalp. These compositions exhibit good skin 25 tolerance.

The invention therefore relates to a cosmetic composition comprising, as an active ingredient, an extract of at least one bacterium from the family 30 *Pseudomonadaceae*, in combination with an excipient which is acceptable in cosmetology.

The excipients present in the composition of the invention are usual excipients. They are excipients 35 which are compatible with use on the skin, on the scalp and/or on the hair.

IN ACCORDANCE WITH THE FILING

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In the compositions used according to the invention, the bacterial extracts are generally present in a proportion of 0.0005% to 5%, for example of 0.001% to 2%, and in particular of 0.01% to 2%, by weight of 5 bacterial solids, relative to the weight of the composition.

These compositions may contain the bacterial extract in the form of dispersions (in particular emulsions) in a suitable vehicle, such as for example water, organic solvents, fatty substances including oils, and mixtures thereof.

The compositions may in particular be in the form of 15 water/alcohol or oil/alcohol lotions, of gels, of emulsions with a liquid consistency, of creams, of solid sticks or of vesicular dispersions. These compositions may be prepared according to the usual methods. They contain the ingredients and vehicles 20 which make it possible to provide them in particular in one of the forms which have just been mentioned. They may contain, besides the bacterial extracts, other active ingredients, such as for example substances which absorb ultraviolet, conventional moisturizers, 25 free-radical scavengers, antioxidants, thermal spring water, such as the water from the thermal springs of La Roche-Posay, emollients or other usual ingredients, such as preserving agents, fragrances, etc. Such ingredients, and also the use thereof, are known and 30 will not be described further here.

The thermal spring water optionally used in the composition of the invention is in particular thermal spring water which has cosmetic properties beneficial 35 for the skin. For example, La Roche-Posay (France) thermal spring water, which is rich in selenium, in particular has protective properties against the deleterious effects of UVA radiation on the skin, and

IN ACCORDANCE WITH THE FILING

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also has antioxidant properties which promote the survival of fibroblasts exposed to UVB radiation. La Roche-Posay thermal spring water therefore constitutes an advantageous active ingredient, in particular in the cosmetic products intended to be used during or after exposure of the skin to sunlight.

A subject of the invention is also a cosmetic treatment method for combating ageing of the skin, characterized in that a composition as defined above is applied to the skin or to the scalp. This composition is applied according to the usual methods.

The following examples illustrate the invention. In these examples, the percentages are percentages by weight.

EXAMPLES

EXAMPLE 1: Culturing *Pseudomonas vesicularis* and *Pseudomonas maltophilia*

The *Pseudomonas vesicularis* strain cultured was obtained from the ATCC (ATCC 11426).

The *Pseudomonas maltophilia* strain is the ATCC 13637 strain.

The bacteria are cultured in Difco Nutrient Broth 003 culture medium(Medium 3 ATCC). The pH of the medium is adjusted to 7.15 before sterilization at 121°C for at least 20 minutes.

The culturing is carried out at 26°C with shaking (100 rpm), ensuring a dissolved oxygen content at least equal to 15%.

After culturing for 24 hours, the biomass is harvested by centrifugation.

IN ACCORDANCE WITH THE FILING

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The biomass may be stabilized by heating in an autoclave, lyophilized, frozen and/or ground.

It is also possible, if desired, to acetylate the  
5 primary and secondary amine groups, totally or partially, via the action of acetic anhydride.

EXAMPLE 2: Cream

10 This cream corresponds to the following composition:

	- Lyophilizate based on <i>Ps. vesicularis</i>	
	obtained according to Example 1 .....	0.05%
15	- Carbomer 940*.....	0.30%
	- Triethanolamine .....	0.30%
	- Stearic acid .....	3.00%
	- Cetyl alcohol .....	2.00%
	- Self-emulsifiable glycerol monostearate .....	3.00%
	- Soya bean oil .....	10.00%
20	- Lanolin alcohol .....	2.00%
	- Isopropyl myristate .....	4.00%
	- Cetearyl 2-ethylhexanoate .....	4.00%
	- Perhydrosqualene .....	3.00%
	- Paraffin .....	2.00%
25	- Glycerol .....	3.00%
	- Preserving agents .....	0.30%
	- La Roche-Posay thermal spring water** .....	15.00%
	- Purified water, q.s. for .....	100.00%

\* Carbomer 940: commercial brand denoting a  
30 crosslinked polyacrylic acid

\*\*La Roche-Posay spa centre (France)

The lyophilizate based on *Pseudomonas vesicularis* may be replaced with a lyophilizate based on *Pseudomonas maltophilia*.

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In a similar manner, a cream containing 0.01% of *Pseudomonas vesicularis* lyophilizate and 0.05% of *Pseudomonas maltophilia* lyophilizate was prepared.

5 To prepare this cream, the aqueous phase containing the glycerol, the preserving agents and the water is heated to 80°C; the Carbomer 940 is dispersed therein, followed by neutralization with triethanolamine. The fatty phase, heated and homogenized at 80°C, is introduced into the aqueous phase, with vigorous stirring. The lyophilizate of Example 1 is dispersed in 10 g of water and introduced, at 40°C, into the cream with stirring. The entire mixture is cooled to ambient temperature.

15 This cream is applied to the skin of the face and of the neck once or twice a day. It improves the appearance of dry skin. It also makes it possible to improve skin tonicity.

20 **EXAMPLE 3: Milk for the skin**

This milk has the following composition:

25	- Lyophilizate of <i>Ps. vesicularis</i> obtained according to Example 1 .....	0.10%
	- Self-emulsifiable glyceryl monostearate .....	3.00%
	- Petroleum jelly .....	1.50%
	- Liquid petroleum jelly .....	2.50%
30	- Rice bran oil .....	1.50%
	- Volatile silicone oil .....	5.00%
	- Karite butter .....	3.00%
	- Carbomer 940 .....	0.20%
	- Triethanolamine .....	0.20%
35	- Xanthan gum .....	0.10%
	- Glycerol .....	3.00%
	- Fragrance .....	0.10%
	- Preserving agents .....	0.30%

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-Water, q.s. for ..... 100.00%

This milk is prepared in a similar way to that described in Example 2.

5

When applied to the skin after exposure to sunlight, it has soothing properties.

When applied to the skin of the face, this milk decreases the effect of accelerated ageing of the skin observed in particular in individuals who are repeatedly exposed to sunlight.

**EXAMPLE 4: Cream**

15

An emulsion having the following composition was prepared according to the same procedure as in Example 2:

20

- Lyophilizate of *Ps. vesicularis* obtained according to Example 1 ..... 0.10%  
- Self-emulsifiable base ..... 20.00%  
- Codex liquid petroleum jelly ..... 5.00%  
- Glycerol ..... 5.00%  
25 - Aluminium stearate ..... 0.50%  
- Dipotassium EDTA ..... 0.05%  
- Magnesium sulphate ..... 0.70%  
- Preserving agents ..... 0.20%  
- Antioxidants ..... 0.05%  
30 - Fragrance ..... 0.30%  
- Water, q.s. for ..... 100.00%

In the above formulation, the *Pseudomonas vesicularis* lyophilizate may be replaced with a *Pseudomonas maltophilia* lyophilizate. A mixture of the two lyophilizates may also be used.

The self-emulsifiable base comprises:

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- Mineral oil
- Codex petroleum jelly
- Ozokerite
- Glyceryl oleate
- 5 - Liquid lanolin.

This cream, when applied to the skin, makes it possible to decrease the effects of ageing of the skin and/or of photoageing of the skin. It also makes it possible to  
10 improve the degree of moisturization of the skin of elderly individuals.

**EXAMPLE 5: Antisun emulsion**

15 This emulsion makes it possible to protect the skin against ultraviolet rays. It corresponds to the following formula:

	- Lyophilizate of Example 1 .....	1.00%
20	- Stearic acid .....	3.00%
	- Cetyl alcohol .....	1.50%
	- Self-emulsifiable glyceryl monostearate .....	3.00%
	- Sunflower oil .....	8.00%
	- Polyacrylamide .....	3.00%
25	- Octyl methoxycinnamate .....	4.00%
	- Triethanolamine salt of benzene-1,4-di-(3-methylidene)-10-camphosulphonic acid (Mexoryl SX) .....	2.60%
	- Glycerol .....	5.00%
30	- Tocopherol .....	2.00%
	- Preserving agents .....	0.30%
	- Ethylenediaminetetramethylene phosphonate (pentasodium salt) .....	0.10%
	- Purified water, q.s. for .....	100.00%

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#### **EXAMPLE 6: Elastase inhibition test**

The test is carried out using elastase isolated from human leukocytes.

5

The test is performed according to the method described by E.O. Adeyemi et al., J . Pharm. Pharmacol., 42:487-490 (1990). The tests are carried out with a lyophilizate obtained as described in Example 1.

10

The lyophilizate originating from the *Pseudomonas maltophilia* culture, at a concentration of 0.05 g/l, decreases the elastase activity by 36%. At a concentration of 0.1 g/l, the lyophilizate originating from the *Pseudomonas vesicularis* culture decreases the elastase activity by 33% and the lyophilizate originating from a *Pseudomonas maltophilia* culture decreases the elastase activity by 53%.

15

**EXAMPLE 7: Inhibitory effect on hyaluronidase activity**

The test is carried out according to the conventional method described in Worthington Enzyme Manual, Enzymes and related biochemicals, Worthington Biochemical Corps., Freehold, New Jersey 07728, USA (1993).

25

The bacterial lyophilizate studied is a *Pseudomonas vesicularis* lyophilizate. It is dissolved in 0.1 M phosphate buffer, pH 5.3.

30

The reagents used are hyaluronic acid (Sigma H-1876) and Sigma hyaluronidase type IV-S (H-3884). The hyaluronic acid and hyaluronidase are mixed in phosphate buffer so as to obtain a solution containing 0.6 g/l of hyaluronic acid and 0.25 g/l of hyaluronidase.

The mixture is left to incubate for 15 minutes at 37°C.

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A solution of bovine albumin at 1% in a 0.5 M acetate buffer, pH 4.2, is then added so as to precipitate the hyaluronic acid.

- 5 The amount of nondegraded hyaluronic acid is then measured by measuring light absorption at a wavelength of 540 nm.

10 The *Pseudomonas vesicularis* lyophilizate, at a concentration of 0.1%, inhibits the hyaluronidase activity by 30%.

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CLAIMS

1. Use of an extract of at least one bacterium from the family Pseudomonadaceae, as a cosmetic agent for combating ageing of the skin, including photoageing, for improving the appearance and tonicity of dry skin, for preserving or improving skin elasticity, and/or for improving the appearance of skin which exhibits a local inflammatory reaction, said extract consisting either of the biomass obtained after culturing said bacterium, optionally followed by purification, grinding, at least partial dehydration and/or sterilization, or of a fraction or a derivative, obtained by chemical modification of certain functional groups, or said biomass; said fraction or said derivative having elastase activity inhibition and/or hyaluronidase activity inhibition properties.
2. Use according to Claim 1, in which said bacterium belongs to the genus *Pseudomonas*.
3. Use according to Claim 2, in which said bacterium is chosen from *Pseudomonas vesicularis* and *Pseudomonas maltophilia*.
4. Use according to any one of the preceding claims, in which said extract consists of the bacterial biomass obtained after culturing the bacterium, said biomass optionally being ground and/or at least partially dehydrated.
5. Use according to any one of the preceding claims, in which said extract is applied in the form of a composition containing a proportion of 0.0005% to 5% by weight of bacterial solids relative to the total weight of the composition.

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6. Use according to the preceding claim, in which said proportion is within the range of 0.001% to 2% by weight.

5 7. Cosmetic treatment method intended to combat ageing of the skin, including photoageing, to improve the appearance and tonicity of dry skin, to preserve or improve skin elasticity, and/or to improve the appearance of skin which exhibits a local inflammatory reaction, said method comprising the step consisting in applying an extract of at least one bacterium from the family Pseudomonadaceae to the skin or to the scalp, said extract either consisting of the biomass obtained after culturing said bacterium, optionally followed by purification, grinding, at least partial dehydration and/or sterilization, or of a fraction or a derivative, obtained by chemical modification of certain functional groups, of said biomass, said fraction or derivative having elastase activity inhibition and/or hyaluronidase activity inhibition properties.

25 8. Method according to the preceding claim, having at least one of the following characteristics:

- the bacterium belongs to the genus *Pseudomonas*;
- the bacterium belongs to the species *Pseudomonas vesicularis* or *Pseudomonas maltophilia*;
- said extract consists of the bacterial biomass obtained after culturing the bacterium, said biomass optionally being ground and/or at least partially dehydrated;
- said extract is applied in the form of a composition containing a proportion of 0.0005% to 5%, and in particular of 0.001 to 2%, by weight of bacterial solids relative to the total weight of the composition.

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9. Cosmetic composition comprising, as an active ingredient, an extract of at least one bacterium from the family *Pseudomonadaceae*, in combination with an excipient which is acceptable in cosmetology, said extract consisting either of the biomass obtained after culturing said bacterium, optionally followed by purification, grinding, at least partial dehydration and/or sterilization, or of a fraction or a derivative, obtained by chemical modification of certain functional groups, of said biomass, said fraction or said derivative having elastase activity inhibition and/or hyaluronidase activity inhibition properties.

15  
10. Composition according to Claim 9, having at least one of the following characteristics:  
- the bacterium belongs to the genus *Pseudomonas*;  
- the bacterium belongs to the species *Pseudomonas vesicularis* or *Pseudomonas maltophilia*;  
- said extract consists of the bacterial biomass obtained after culturing the bacterium, said biomass being at least partially dehydrated;  
20  
25 - said composition contains a proportion of 0.0005% to 5%, and in particular of 0.001 to 2%, by weight of bacterial solids relative to the total weight of the composition.

10/049552

JC13 Rec'd PCT/PTO 14 FEB 2002

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**ABSTRACT**

Limited company named:

**L'OREAL**

For:

"Use of bacterial extracts from the family  
Pseudomonadaceae as cosmetic agents"

---

Use of an extract of bacterium from the family Pseudomonadaceae in the production of cosmetic compositions in particular for combating ageing of the skin.

RECEIVED APRIL 16 APR 2002  
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Docket No.:

10/049552

DECLARATION AND POWER OF ATTORNEY  
UNDER 35 USC §371(c)(4) FOR  
PCT APPLICATION FOR UNITED STATES PATENT

As a below named inventor, I hereby declare that:  
my residence, post office address and citizenship are as stated below under my name;

I verily believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought, namely the invention entitled Use of bacterial extracts from the family Pseudomonadaceae as cosmetic agents

described and claimed in international application number PCT/FR99/02043 filed on August 26, 1999

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations §1.56.

Under Title 35, U.S. Code §119, the priority benefits of the following foreign application(s) filed by me or my legal representatives or assigns within one year prior to my international application are hereby claimed:

The following application(s) for patent or inventor's certificate on this invention were filed in countries foreign to the United States of America either (a) more than one year prior to my international application, or (b) before the filing date of the above-named foreign priority application(s):

I hereby appoint the following as my attorneys of record with full power of substitution and revocation to prosecute this application and to transact all business in the Patent Office:

James A. Oliff, Reg. No. 27,075; William P. Berridge, Reg. No. 30,024;  
Kirk M. Hudson, Reg. No. 27,562; Thomas J. Pardini, Reg. No. 30,411;  
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Joel S. Armstrong, Reg. No. 36,430; Christopher W. Brown, Reg. No. 38,025; and  
Richard E. Rice, Reg. No. 31,560.

ALL CORRESPONDENCE IN CONNECTION WITH THIS APPLICATION SHOULD BE SENT TO OLIFF & BERRIDGE, PLC, P.O. BOX 19928, ALEXANDRIA, VIRGINIA 22320, TELEPHONE (703) 836-6400.

I hereby declare that I have reviewed and understand the contents of this Declaration, and that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1	Typewritten Full Name of Sole or First Inventor	Richard	MARTIN
2	Inventor's Signature:	Given Name <u>Richard</u>	Family Name <u>Martin</u>
3	Date of Signature:	Month <u>4</u>	Day <u>3</u>
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Note to Inventor: Please sign name on line 2 exactly as it appears in line 1 and insert the actual date of signing on line 3.

IF THERE IS MORE THAN ONE INVENTOR USE PAGE 2 AND PLACE AN "X" HERE   
(Discard this page in a sole inventor application)

1	Typewritten Full Name of Joint Inventor		
2	Inventor's Signature:		
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		City	Year
	Citizenship:	State or Province	
	Post Office Address: (Insert complete mailing address, including country)		

Note to Inventor: Please sign name on line 2 exactly as it appears in line 1 and insert the actual date of signing on line 3.

This form may be executed only when attached to the first page of the Declaration and Power of Attorney of the application to which it pertains.